REMARKS

This Amendment cancels claims 23, 24, 29, 31 and 32, adds new claims 33 and 34, and amends claims 17, 18, 20-22, 25-28 and 30. The features of claim 29(i) have been incorporated into claim 17(i), while the features of claim 24 have been incorporated into claim 17(ii). The amendment of claims 26 and 30 merely changes their dependency. The comparison step/reference amount features of claim 27 are supported by page 16, lines 17-31 and Fig. 11. New claims 33 and 34 correspond to claims 25 and 26. Claims 17-22, 25-28, 30, 33 and 34 are pending.

This Amendment overcomes the 35 U.S.C. § 112, second paragraph, rejection of claims 17-32. Independent claims 17 and 27 has been amended to positively recite method steps and provide antecedent basis for subsequent claim terms. Claims 18 and 22 have also been amended to eliminate lack of antecedent basis objections. Claims 30 and 34 now recite "first" and "second" binders to clearly distinguish one from the other. One of ordinary skill in the art would understand the metes and bounds of the claimed methods. Reconsideration and withdrawal of the indefiniteness rejection of claims 17-32 are respectfully requested.

The 35 U.S.C. § 102(b) rejection of claims 17, 23, 31 and 32 over PCT Patent Publication WO 00/54806 to Overgaard et al. is traversed. The claimed bioaffinity assay determines free PAPP-A present in a sample by either

i) by exposing said sample to a first binder which binds total PAPP-A and to a second binder which binds only PAPP-A complexed to proMBP and detecting said first and second binders, and calculating the difference between measured total PAPP-A and measured PAPP-A complexed to proMBP, or

ii) by a direct bioaffinity assay measuring only free PAPP-A, by making PAPP-A complexed to proMBP non-capable of participating in the bioaffinity reaction in which said sample is exposed to a binder which binds total PAPP-A.

Overgaard et al. fails to disclose these steps of the claimed assay. Reconsideration and withdrawal of the anticipation rejection of claims 17, 23, 31 and 32 over Overgaard et al. are respectfully requested.

The 35 U.S.C. § 102(e)(2) rejection of claims 17, 23, 31 and 32 over U.S. Patent No. 7,115,382 to Overgaard et al. is traversed. The claimed bioaffinity assay determines free PAPP-A present in a sample by either

i) by exposing said sample to a first binder which binds total PAPP-A and to a second binder which binds only PAPP-A complexed to proMBP and detecting said first and second binders, and calculating the difference between measured total PAPP-A and measured PAPP-A complexed to proMBP, or

ii) by a direct bioaffinity assay measuring only free PAPP-A, by making PAPP-A complexed to proMBP non-capable of participating in the bioaffinity reaction in which said sample is exposed to a binder which binds total PAPP-A.

Overgaard '382 also fails to disclose these steps of the claimed assay. Reconsideration and withdrawal of the anticipation rejection of claims 17, 23, 31 and 32 over Overgaard '382 are respectfully requested.

The 35 U.S.C. § 102(b) rejection of claims 17, 23, 27, 28, 31 and 32 over U.S. Patent No. 6,500,630 to Conover et al. is respectfully traversed. A feature of the claimed methods is the analysis of free PAPP-A, rather than complexed PAPP-A, in a sample and its use as a marker for acute coronary syndrome.

Conover et al. fails to disclose the analysis and use of free PaPP-A as a marker for acute coronary syndrome. One of ordinary skill in the art would not interpret Conover et al. to disclose the

use of monoclonal antibodies specific for PAPP-A not complexed with proMBP for the detection of uncomplexed PAPP-A in a sample of patients to be diagnosed for acute coronary syndrome.

One of ordinary skill in the art would consider the wording in Conover et al. at col. 4, lines 47-49 to be unclear because "Monoclonal antibodies having specific binding affinity for PAPP-A, but not for PAPP-A/proMBP complexes, can be produced through standard methods", is subject to at least two alternative interpretations:

Alternative 1: the monoclonal antibody (mab) has specific binding affinity for PAPP-A, but it does not have specific binding affinity for the PAPP-A/proMBP complex. This means that the mab in fact can also recognize the PAPP-A/proMBP complex although the mab is not specific for the complex. If both PAPP-A/proMBP complex and free PAPP-A are present in a sample, such a mab will detect them both, i.e. total PAPP-A.

Alternative 2: the mab has specific binding affinity for PAPP-A, and the mab is not able to detect the PAPP-A/proMBP complex (i.e. the mab is directed to an epitope region of PAPP-A that would be occupied by proMBP if PAPP-A exists in PAPP-A/proMBP complex). This means that the mab detects exclusively free PAPP-A.

The inventors have studied the Conover et al. Examples to determine which alternative is correct, and have concluded the Conover et al. experimental arrangement will lead to detection of total PAPP-A. See Appendix 1, attached hereto. Thus Col. 4, lines 47-49 is properly interpreted to mean the monoclonal antibodies will detect total PAPP-A in accordance with alternative 1.

Col. 7 of Conover et al., cited by the Patent Office, does not disclose an assay for PAPP-A activity which inherently measures the free active form, in which the enzyme is captured with an antibody and reacted with substrate. This is because PAPP-A activity relates theoretically to any form of PAPP-A which is enzymatically active. Uncomplexed intact PAPP-A is expected to be enzymatically active but it is generally known that many proteases, while appearing in a free form, may still lack enzymatic activity due to e.g having internal cleavages or representing a proform of the protease [Chua et al., 275 J. Biol. Chem. 2000 5131-5 (2000); Borgoño et al., 2 Mol. Cancer Res. 257-80 (2004); Wu et al, 58 Prostate 345-53 (2004)]. The determination of activity heavily relies on the specificity of substrates used. Immunoassays can be designed to measure free PAPP-A no matter whether it is

proteolytically active or not, whereas protease assays only determine proteolytically active forms. In case PAPP-A is partially complexed with proMBP, meaning that only one proMBP subunit is complexed with two PAPP-A subunits [Overgaard et al., 275 J. Biol Chem. 31128-33 (2000)], the enzyme activity assays (but not immunoassays) can give misleading results. Importantly, the clinical value of PAPP-A in acute coronary syndrome has only been revealed by immunoassays and not by PAPP-A activity assays. Clinical data to prove the value of activity assays in ACS are simply lacking. Furthermore, enzymatic assays are known to be inherently less sensitive than immunoassays employing a non-competitive (sandwich) assay design with a reporter system of high specific activity. Methods for detecting the ACS-related increases in uncomplexed PAPP-A need to be both extremely sensitive and rapid (due to the acute nature of the disease), and enzyme activity assays can satisfy neither of these requirements.

Reconsideration and withdrawal of the anticipation rejection of claims 17, 23, 27, 28, 31 and 32 over Conover et al. are respectfully requested.

The claimed methods are nonobvious to one of ordinary skill in the art. Neither of the references disclose or suggest the use of

free PAPP-A as a marker for acute coronary syndrome. The inventors have demonstrated "the delta value" (i.e. free PAPP-A) is, compared to total PAPP-A, a very good marker and useful to exclude false negative PAPP-A results. See page 16, lines 17-31 and Fig. 11 of the application. The advantages provided by the present invention could not have been predicted by those of ordinary skill in the art.

A Supplemental Information Disclosure Statement is attached which submits the documents discussed above and in the attached Appendix.

It is believed this application is in condition for allowance. Reconsideration and withdrawal of all rejections of claims 17-32, and issuance of a Notice of Allowance directed to claims 17-22, 25-28, 30, 33 and 34, are respectfully requested. The Examiner is urged to telephone the undersigned should he believe any further action is required for allowance.

It is not believed any fee is required for entry and consideration of this Amendment. Nevertheless, the Commissioner is

authorized to charge Deposit Account No. 50-1258 in the amount of any such required fee.

Respectfully submitted,

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Enclosures:
Information Disclosure Statement
Appendix

Appendix

Inventors' Comments on U.S. Patent No. 6,500,630

The method described in Example 1 of U.S. Patent No. 6,500,630 for measuring PAPP-A in blood (plasma) samples obtained from control subjects and sick patients was a sandwich biotin-tyramide amplified ELISA, where PAPP-A polyclonal antibodies were used for capture and a combination of monoclonal antibodies was used for detection. The polyclonal antibodies were in fact made with highly purified PAPP-A/ProMBP [Christiansen et al., "Quantification and characterization of pregnancy-associated complexes of angiotensinogen and the proform of eosinophil major basic protein in serum and amniotic fluid," 46 Clin Chem 1099-1105 (2000)]. Monoclonal antibodies were 234-2, 234-3, 234-4, 234-5 and 234-6, which were all raised with PAPP-A/proMBP complex purified from term serum (Qin et al., "Double-monoclonal immunofluorometric assays for pregnancy-associated plasma protein A/proeosinophil major basic protein (PAPP-A/proMBP) complex in first-trimester maternal serum screening for Down syndrome, 43 Clin Chem 2323-2332 (1997)]. Under both native and denatured, reduced conditions, Western blots have demonstrated that these antibodies, namely 234-2, 234-3, 234-4, 234-5 and 234-6 react with the PAPP-A part of the PAPP-A/proMBP complex and not the proMBP part (Qin, "Maternal serum screening for Down syndrome in the first trimester with special emphasis on

pregnancy associated plasma protein A," PhD thesis 144pp (University of Turku Turku, Finland 1998).

PAPP-A isolated from pregnancy serum is a disulfide-bound 2:2 complex with the proform of eosinophil major basic protein (proMBP) in which each PAPP-A subunit is connected to a proMBP subunit by 2 disulfide bonds. The antibodies, regardless of being polyclonal or moloclonal, reactive with the PAPP-A sites blocked by proMBP cannot be raised by immunization with PAPP-A/proMBP extracted from pregnancy serum. Resultant antibodies are either reactive with the proMBP-unblocked part of PAPP-A unit or the proMBP part of PAPP-A/proMBP complex.

The ELISA method used in U.S. Patent No. 6,500,630 thus detects total PAPP-A molecules, which means both free PAPP-A and proMBP-complexed PAPP-A molecules are detected with the method.

In the immunohistochemical staining, monoclonal antibody 234-5 was used. As the antibody binds to the proMBP-unblocked part of PAPP-A unit, the positive staining reflects the distribution of both free and proMBP-complexed PAPP-A molecules in the plaque tissue.